International Journal of Hospital Research 2018, 7(1): x-x
http://ijhr.iums.ac.ir

doi 10.15171/ijhr.2018.xx

Research Article

IJHR

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Investigating the Prevalence of Enteric Opportunistic Parasitic Infections Among Cancer Patients of a Teaching Hospital

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Abstract

Background and Objectives: Cancer patients treated with chemotherapy and other immunosuppressive drugs are always prone to various infections including opportunistic parasites. Since detection of infections in immunocompromised patients are frequently imperfect and the usual symptoms such as pyrexia are missing or hidden due to leukopenia, the importance of detection of opportunistic parasitic infections is well justified. Therefore, we aimed in this study to investigating the prevalence of enteric opportunistic parasitic infections among cancer patients of a selected teaching hospital affiliated to Iran University of Medical Sciences.

Methods: This descriptive-analytical cross-sectional study was carried out on 150 cancer patients admitted to the oncology ward of a selected teaching hospital affiliated to Iran University of Medical Sciences in Iran from July 2016 to December 2017. Patients for this study were chosen by simple random selection method. Fecal samples from these patients were gathered and intestinal parasites were identified using direct wet mount, formalin-ether, chromotrope 2R staining and acid-fast staining methods. The obtained data from patients were analyzed using analysis of variance (ANOVA), *t* test and chi-square test. All statistical analyses were carried out through SPSS version 17.0.

Findings: Among 150 samples investigated with direct wet mount method, 23 were reported positive for parasites with the most frequent parasite being *Blastocystis* (14%). Investigation of slides stained by hot acid-fast method revealed no cases contaminated by *Cryptosporidium* spp. or *Isospora belli*, yet in fecal samples stained with chromotrope 2R method 9 *Microsporidia* sp. infection cases were reported.

Conclusions: It was believed that due to immunosuppressive effect of chemotherapeutic agents, the treated patients are more prone to opportunistic infections. Contrary to this belief our study showed lower prevalence of infections in these patients which could be related to more prophylactic drug use that are antibacterial as well as antiparasitic.

Keywords: Opportunistic parasites, Cancer, Chemotherapy, Teaching hospital

Background and objectives

Infection diseases, especially opportunistic infections, are one of the major complications among immunocompromised patients and patients treated with immunosuppressive agents. These groups involve a vast range of patients including cancer patients, organ transplant recipients and patients treated with immunosuppressive drugs and chemotherapy are more susceptible to various infections and endure severe complications.[1,2] For instance, chemotherapy in patients suffering from malignancy may result in life-threatening toxoplasmosis or corticosteroid prescription to treat ulcerative colitis may lead to acute amoebiasis in infected patients. A body of evidence suggest that some parasites such as *Strongyloides stercoralis* and *Trichomonas vaginalis* are more common in immunocompromised settings. Patients with acquired immune deficiency syndrome are more prone to opportunistic infections such as toxoplasmosis, cyclosporiasis, isosporiasis and severe strongyloidiasis while prevalence of non-opportunistic infections such as amoebiasis, giardiasis

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and schistosomiasis is unchanged.[3-5]

Furthermore, contamination with enteric parasites is a major health issue in human populations especially in developing countries which are usually located in tropical regions. An estimated 3.5 billion people are affected and approximately 450 million individuals currently suffer from these infections.[6] Usual signs and symptoms of parasitic infections in humans include malabsorption, abdominal pain, diarrhea, constipation, anorexia, nausea, fever, stomach cramps and delayed growth in children and it should be noted that these conditions impose heavy costs on governments.[7,8]

Prevalence of parasitic infections in cancer patients has been reported in some studies; although the results are sometimes different the consensus is that the prevalence of infectious parasites is higher among cancer patients treated with immunosuppressive drugs compared to healthy control group.[9-12]

Kazemi et al reported the overall frequency of intestinal parasites among immunosuppressed patients undergoing chemotherapy in Khuzestan to be 18.5%.[13] Abdel-Magied et al studied the prevalence of intestinal parasites in cancer therapy recipients and reported 85.5% as the frequency of protozoa among the patients showing the clinical symptom of concurrent diarrhea.[14]

Although there is no significant statistical difference between different immunodeficiencies and prevalence of enteric parasites, strong correlation is seen between parasite presence and clinical signs in immunocompromised patients.[15]

Since diagnosis of parasitic infections in immunocompromised patients is commonly fallible and the usual symptoms and signs such as fever are either hidden or absent due to leukopenia, the necessity for detection of infectious parasites in these groups is well-recognized. On the other hand, growth and development of medical sciences and enhancement of cancer detection methods and the resulted promotion of chemotherapy and radiotherapy treatments as well as increased organs transplantations have elevated the number of immunocompromised patients in countries.[16,17] In this regard, the aim of this study was to investigate the prevalence of enteric opportunistic infections in cancer patients of a selected teaching hospital affiliated to Iran University of Medical Sciences.

Methods

This descriptive-analytical cross-sectional study was carried out on 150 cancer patients admitted to the oncology ward of a selected teaching hospital affiliated to Iran University of Medical Sciences in Iran from July 2016 to December 2017.

A number of 150 patients were selected using random selection method to participate in this study. Fecal specimens were collected once from each patient and preserved in 10% formalin. Samples were then shipped to parasitology laboratory of Iran university of medical sciences for further microscopic investigations.

The patients were categorized into 4 age groups: ≤20 years, 21-40 years, 41-60 years and >60 years. Age, gender, cancer type and chemotherapy regimens were individually recorded and analyzed.

Microscopic Examination of Specimens

The samples were concentrated using formalin-ether technique, and the resulting sediment was evaluated by the following three methods: Wet Mount technique (To detect protozoan cysts and helminths ova), Hot acid-fast staining (to detect *Cryptosporidium* spp. and *Isospora belli*) and chromotrope 2R staining (to detect *Microsporidia* sp).

1. Wet Mount Method

Ten milliliters of 10% formalin was first added to 1 g of fecal specimen and mixed with a wooden applicator to gain a cloudy suspension. Mesh gauze was then placed on top of the conical centrifuge tube and the suspension was filtered through the gauze into the tube. 3-4 mL of ether was added to the tube and after shaking with hand it was centrifuged at about 2000 rpm for 2 minutes. Supernatant was then decanted and the remaining sediment was gathered to prepare wet mount smear.[18]

2. Hot Acid-Fast Staining

30-50 μL of sediment obtained from formalin-ether method was transferred to a microscope slide using sampler. The specimen was dried before fixation with methanol. Carbol fuchsin was applied to the slide to cover the whole surface and after 5 minutes it was heated on a flame so that residual liquid evaporates from the surface. Slide was then left alone for 5 to 7 minutes to cool and absorb the stain. After rinsing with distilled water, acid-alcohol was applied to the slide as decolorizer for 10 seconds and then rinsed again. Methylene blue (background stain) was applied to the slide for one minute and after rinsing and drying the slide was sent for microscopic imaging.[19,20]

3. Chromotrope 2R Staining

Slides prepared with sediments obtained from formalin-ether method were fixed with methanol. These slides were then held in chromotrope stain for 90 minutes. After destaining with acid-alcohol for 10 seconds, dewatering was carried out with 95% alcohol twice for 5 minutes and

again with pure alcohol for 10 minutes. Finally, slides were flooded in xylene for 10 minutes and then mounted using Canada balsam.[21,22]

The consent form containing the information about the nature of this study was prepared in a non-academic and understandable language and all the participants were educated about the aim of the study. The participants were free to willingly decide to take part in the study. All the information regarding to individual patients were kept confidential and the results were published categorically without revealing patients' names or data.

Statistical Analysis

The normal distribution of data was evaluated using the Kolmogorov–Smirnov test. The difference in means was analyzed by one-way analysis of variance (ANOVA) and *t* test. The comparison between categorical variables was performed using chi-square test. Data were analyzed using SPSS version 17 statistical software. The significance level of 0.05 was considered.

Results

Among 150 studied patients 81 (54%) were male and 69 (46%) were female participants and their age-span ranged from 16 to 81 years old with an average of 45.2 years. Frequencies of samples in different age groups were as follows:≤20, , 38% (41-60) and (>60. The highest frequency was in 40-60 age group (38%) while the least frequency was for the age group under 20 (13%) (Table 1). There was no statistically significant difference between prevalence of opportunistic enteric parasites among male and female sex or among different age groups ($P>0.05$).

Wet Mount Results

Among 150 fecal samples studied directly using formalin-ether technique 23 cases (15.3%) were reported positive with highest frequency of 21 (14%) cases positive for *Blastocystis* sp. which was accompanied by *Iodamoeba butschlii* in 1 case (Table 2). Contamination among female and male cases was 13 (16%) and 10 (14.5%) respectively. Highest contaminated population in age groups was also in 21-40 years old

Hot Acid-Fast Staining Results

Stained slides using hot acid-fast technique revealed no oocytes of *Cryptosporidium* spp. or *Isospora belli* among the patients, hence the frequency of these coccidian parasites was reported zero.

Chromotrope 2R Staining Results

Among 150 samples stained using chromotrope 2R, 9 (6%) were reported positive. These cases were all contaminated with *Microsporidia* sp. and consisted of 3 males (2%) and 6 females (4%) the majority of which were in 40-60 years age group (Table 2). Still, no significant relationship was observed between sex and contamination with *Microsporidia* sp. ($P>0.05$).

Table 1. Association Between Sex and Age Variables With Positive Cases for Intestinal Protosoa in Cancer Patients

Variables	No. (%)	Positive Patients No. (%)	P Value
Sex			0.087
Male	81 (54)	13(16)	
Female	69 (46)	19(27)	
Age group (y)			0.537
≤ 20	20 (13)	4 (20)	
21-40	41 (27)	12 (29)	
41-60	57 (38)	10 (18)	
> 60	32 (22)	6 (19)	

Table 2. Frequency Distribution of Different Intestinal Opportunistic Parasite Types in Pations

Infection Frequency	Diagnostic Method		
	Formalin-ether	Acid-fast Staining	Chromotrope 2R
	No. (%)	No. (%)	No. (%)
Blastocystis sp.	20 (13.34)	0 (0)	0 (0)
Giardia lamblia	1 (0.66)	0 (0)	0 (0)
Entamoeba coli	1 (0.66)	0 (0)	0 (0)
Blastocystis sp. + *Iodamoeba butschlii*	1 (0.66)	0 (0)	0 (0)
Cryptosporidium spp.	0 (0)	0 (0)	0 (0)
Isospora beli	0 (0)	0 (0)	0 (0)
Microsporidia sp.	0 (0)	0 (0)	9 (6)
Negative	127 (84.68)	150 (100)	141 (94)
Total	150 (100)	150 (100)	150 (100)

Investigation of Gastrointestinal Symptoms Among Cancer Patients

About 68.6 percent of our studied cases showed no gastrointestinal symptoms and the majority of symptoms were relating to diarrhea and abdominal pain. No significant relation was observed among age and sex groups respecting the clinical symptoms ($P>0.05$).

Discussion

Among all fecal samples studied directly using formalin-ether technique and chromotrope 2R, 15.3% and 6% were reported positive, respectively. Stained slides using hot acid-fast technique revealed no parasite. Frequency of intestinal parasites among cancer patients was

21.3% in the current study, 6% of which was attributed to *Microsporidia* sp. that are categorized under fungi in recent classifications.

Various studies had considered the prevalence of parasitic infections among cancer patients with no consistent agreement.[10,12,23] Some studies showed higher intestinal parasite rates among cancer patients treated with immunosuppressive drugs than healthy group.[10,23] Monsef et al reported lower prevalence of intestinal infections in malignancy patients than general population; they suggested it being a consequence of drugs used in chemotherapy.[11] In another study by Menon et al a frequency of 42% was reported positive among children with cancer. Although about 50% of those children were in contact with domestic animals, only 2% were reported positive for *Cryptosporidium* spp.[12]

Kazemi et al investigated the prevalence of intestinal parasitic infections in cancer patients undergoing chemotherapy. Their reported frequency for *Blastocystis huminis*, *Giardia lamblia*, *C. parvum* and *Isospora belli* were 6%, 3.5%, 3% and 0.5% respectively. Difference in reported frequencies between their study and ours could be related to different detection methods[13]; while they used idoine staining and Sheather's floatation method to detect protozoa, formalin-ether sedimentation method, hot acid-fast staining and chromotrope 2R staining were the exploited methods in the current study.

Abdel-Magied et al reported the frequencies of *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica* in cancer therapy recipients with concurrent diarrhea as 36.6%, 30.3% and 27.6%, respectively. Only *Cryptosporidium parvum* and *Entamoeba histolytica* were reported as significantly mixed infections with other parasites in this study.[14] Prevalence of *Giardia lamblia* and *Cryptosporidium* spp. in our study were 0.66% and 0% respectively while mixed infection was only observed in co-occurrence of *Blastocystis* sp. and *Iodamoeba butschlii*. Difference in these studies could be attributed to different therapeutic agents prescribed to those patients and the fact that Abdel-Magied et al studied the patients who presented clinical symptoms of intestinal malfunctions while our sampling group consisted of all cancer patients.

According to previous studies, it was believed that cancer patients who are under chemotherapeutic treatments are more prone to parasitic infections since these drugs are generally immunosuppressive. The same statement is also true for cancer itself considering some cancers modulate immune response and in many cases cancers appear in immunocompromised backgrounds.[24,25] Our result was contradictory to this belief, showing a lower prevalence of intestinal parasites among cancer patients undergoing chemotherapeutic treatments.

This result is consistent with Guarner et al who studied the frequency of intestinal parasites in cancer patients in Mexico.[26] It should be noted that in spite of immunosuppressive effects of chemotherapeutic drugs, these agents could also pose destructive effects on parasite cells and reduce them in those hosts in this manner. Cancer patients are also usually less exposed to infections because of intensive care and reduced mobility. Moreover, 7.28% of samples gathered were watery or soft which could affect the accuracy of detection in those samples and cause errors.

Statistical analysis revealed no significant relation between intestinal parasitic infection prevalence and sex or age ($P>0.05$) which was in disagreement with findings of Azizi et al.[27]Although they could not find a statistical relation between parasitic infections and sex, yet in their study highest contamination frequency was in 21-30 years age population (37.2%) and the lowest frequency was among under 20 age group (12.5%). Chi-square test showed significant difference in 21-30 age group infection rate compared to other age groups. Disagreement in our results with Azizi's could be attributed to different sampling populations which was 150 in our study compared to 600 in their work or it could be a result of different age-spans when grouping. Age groups in our study were ≤20 (20%), 21-40 (29%), 40-60 (18%) and >60 (19%) which showed no significant difference among groups ($P>0.05$).

Since the drugs used in chemotherapy have modulating effects on cellular immunity, they could suppress part of host's immunological function, hence predisposing the host to opportunistic parasites such as *Cryptosporidium* spp., *Isospora belli, Microsporidia* sp. or even giardia. Still in this study no positive *Cryptosporidium* spp. or *Isospora belli* cases were observed while the prevalence of *Microsporidia* sp. and *Giardia lamblia* were 6% and 0.66% respectively. Lower frequencies of these parasites in study are generally in agreement with those studies that suggest chemotherapeutic agents have antiparasitic effects.[28] This question yet remains unanswered that why the same does not apply to other protozoa. For instance, these drugs did not reduce the prevalence of *Blastocystis* sp. among other parasites which needs further studies and investigation. in general, the results of current study and other studies performed in recent years in Iran shows decreasing trends of parasitic infections among immunocompromised patients and patients treated with chemotherapy. In this aspect to minimize contamination and to break the cycle of parasite transfer, some approaches have been proposed to prevent contamination which include: optimizing wastewater systems and providing sanitary drinking

water in rural and urban areas as well as education plans in health centers. Even though the ratios of people infected with intestinal opportunistic infections has not increased in cancer or immunocompromised patients respecting to healthy population, yet the risk of mortality and long range-term adverse effects of these parasites in immunodeficient patients is much higher, emphasizing the need for prophylactic drugs in these patients.

Conclusion

It was believed that due to immunosuppressive effect of chemotherapeutic agents, the treated patients are more prone to opportunistic infections. Contrary to this belief our study showed lower prevalence of infections in these patients which could be related to more prophylactic drug use that are antibacterial as well as antiparasitic. On the other hand, elevated general hygiene standards have lowered parasitic infection prevalence among human populations. Although our study showed decreased frequency of opportunistic parasitic infections among cancer patients treated with immunosuppressive drugs, yet the occurrence of these diseases in immunosuppressed patients could be fatal. Hence, it is advised to exploit detection methods that are specific for opportunistic protozoa in these patients instead of the usual general methods.

Authors' Contributions

SS, TE and HN searched the literature and performed experiments. MR and ARM designed the study and analyzed the data. AB and AM and have participated in drafting the manuscript and supervised the research. TE, SS and AM wrote the final manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

Acknowledgements

The authors would like to thank staff of Firoozgar hospital for their cooperation and their generous help with sampling. We would also like to acknowledge Iran University of Medical Sciences for their financial support of this study.

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Please cite this article as:
Soudeh Salehi , Taher Elmi, Ahmad Reza Meamar, Ali Basi, Amirhossein Mirhosseini1, Hoda Namdari, Mitra Ranjbar. Investigating the Prevalence of Enteric Opportunistic Parasitic Infections among Cancer Patients of a Teaching Hospital. International Journal of Hospital Research 2018, 7(1): x-x.